LR-4795
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RESEARCH ARTICLE   Legume Research- An International Journal, Volume Issue : ()

Biochemical Basis of Resistance in Chickpea (*Cicer arietinum* L.) against Wilt Complex

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10.18805/LR-4795

ABSTRACT

Background: The chickpea wilt complex caused by several soil-borne pathogens, is the most destructive disease of chickpea that causes severe losses in yield worldwide. The development of a resistant variety is the most appropriate approach for managing the disease.

Methods: The present investigation was aimed to find out the resistant sources against the wilt complex of chickpea under pot conditions and studies on biochemical characters responsible for disease resistance during *rabi* 2018-19 and 2019-20 at Anand Agricultural University, Anand, Gujarat.

Result: Among the thirty-seven varieties/germplasm of chickpea, the final disease reaction of two years revealed that two varieties/ germplasm *viz.,* GJG-6 and GAG-0624 showed a resistant reaction and three varieties/ germplasm (GJG-3, Dayadara and Khedbrahma) showed a moderately resistant reaction. The biochemical analysis of the resistant and susceptible varieties/germplasm revealed that healthy roots of resistant and susceptible germplasm showed higher moisture content (85.90-88.42%), while it was lower (74.71-80.22%) in diseased roots. Concerning protein content, the least (32.14%) decrease in protein content was recorded in resistant germplasm, whereas, the highest decrease of 41.79 per cent was recorded in susceptible germplasm. *W.r.t.* to phenol content, diseased roots of resistant and susceptible germplasm contained higher phenol content compared to healthy roots. Similarly, the minimum per cent decrease in total soluble sugars content (32.14%) was observed in resistant germplasm, while it was highest (41.82%) in susceptible germplasm.

Key words: Biochemical characters, Chickpea wilt complex, Fusarium wilt, Phenol, Protein, Screening, Total soluble sugars.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is an important pulse crop of India. It is grown in more than 50 countries and India is the largest producer of chickpea in the world. India occupied about 10.56 million hectares area and total production of 11.23 million tonnes with average productivity of 1063 kg/ ha during 2017-18 (Anonymous, 2018).

Nearly 172 pathogens (67 fungi, 3 bacteria, 22 viruses and 80 nematodes) have been reported infecting chickpea world-wide (Nene *et al.*, 1996). Chickpea wilt complex caused by several soil-borne pathogens is highly destructive disease that causes severe yield losses. In these soil-borne pathogens, Fusarium wilt [*Fusarium oxysporum* Schlech tend. Fr. f. sp. *ciceri* (Padwick) T. Matuo and K. Satõ], black root rot [*Fusarium solani* (Mart.) Sacc.], dry root rot [*Macrophomina phaseolina* (Tassi) Goidanich], wet root rot [*Rhizoctonia solani* Kühn] and collar rot [*Sclerotium rolfsii* Sacc.] are of considerable importance (Nene *et al.*, 1981).

Management of wilt complex of chickpea is difficult to achieve as the pathogens are soil-borne, surviving through resistant structure *i.e.* chlamydospores and sclerotia in the soil for years even in the absence of host and the crop remains susceptible throughout all the growth stages (Haware *et al.*, 1986; Kaiser *et al.*, 1994).

The development of resistant cultivars is the most viable, environmentally safe and economically sound technique for the management of the disease. Further, screening of disease resistant plants can be effective if the biochemical parameters of disease resistance in plants are well understood as the host-pathogen interaction is very complex and diverse in nature. During the survey in major chickpea growing regions of Gujarat, *Fusarium oxysporum* f. sp. *ciceri, Fusarium solani* and *Macrophomina phaseolina* were found predominantly associated with wilt complex infected chickpea plants. Hence, the present investigation was therefore undertaken to find out the resistant sources of chickpea varieties/germplasm against these soil-borne pathogens during *rabi* 2018-19 and *rabi* 2019-20 and then to identify biochemical parameters associated with the resistance.

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How to cite this article: Parmar, H.V. and Gohel, N.M. (2022). Biochemical Basis of Resistance in Chickpea (*Cicer arietinum* L.) against Wilt Complex. Legume Research. DOI: 10.18805/LR-4795.

Submitted: 22-09-2021 **Accepted:** 10-02-2022 **Online:** 07-03-2022

MATERIALS AND METHODS

A total of thirty-seven varieties/germplasm of chickpea including national check JG 62 were screened under pot conditions at the Department of Plant Pathology, B. A. College of Agriculture, AAU, Anand during rabi 2018-19 and 2019-20.

Screening of chickpea varieties/germplasm

The sick pot technique developed by Nene *et al.* (1981) was followed for the experiment. Sterile soil was placed in 30×30 cm earthen pots. Pure cultures of *F. oxysporum* f. sp. *ciceri, F. solani* and *M. phaseolina* were grown on Potato Dextrose Agar (PDA) medium and multiplied on 100 g of 9:1 sand maize meal medium in 250 ml conical flasks for 15 days at 25±1°C. The fungus grown on sand maize meal medium in flasks was added at 100 g/2 kg of soil. Twenty seeds of respective varieties/germplasm of chickpea were sown in the pot. Observations on seedling mortality were recorded up to 30 days after sowing. The final observation on seedling mortality was considered to categorize the chickpea varieties/germplasm (Table 1) into different reactions (Nene *et al.*, 1981).

Seedling mortality was calculated (Pande *et al.,* 2012) as follow:

Seedling mortality (%) =

$$\frac{\text{Number of infected seedlings}}{\text{Total No. of seedlings}} \times 100$$

Biochemical analysis

The effect of biochemical characters was estimated from two of each promising varieties/germplasm representing resistant and susceptible disease reaction from diseased and healthy plant roots. The sampling was done during the morning hours. The samples were then transferred to polythene bags separately and carried to the laboratory in an icebox containing ice cubes to prevent any denaturation of enzymes.

Moisture content was estimated by using the following formula:

Moisture content (%) =

$$\frac{\text{Initial weight} - \text{Oven dry weight}}{\text{Initial weight}} \times 100$$

The protein content (%) was estimated by Lowry *et al.* (1951), phenols (mg/g of fresh weight) were estimated in acetone extract followed by addition of Folin-Ciocalteau reagent using catechol as a standard method (Bray and Thorpe, 1954) and total soluble sugars (mg/g of fresh weight) were determined by following the method suggested by Bhatnagar *et al.* (2006) with some modifications.

All these experiments were done in triplicate in completely randomized design (CRD) and the results were statistically analyzed. The biochemical analyses were carried out at the Department of Biochemistry, BACA, AAU, Anand.

Table 1: Disease reaction categories based on disease incidence/ seedling mortality.

Seedling mortality (%)	Disease reaction
0-10	Resistant (R)
10.1-20	Moderately resistant (MR)
20.1-40	Moderately susceptible (MS)
40.1-100	Susceptible (S)

RESULTS AND DISCUSSION

Screening of chickpea varieties/germplasm

The varieties/germplasm were grouped under different degrees of resistance based on per cent disease incidence. The result presented in Table 2 showed that there were considerable differences among the varieties/germplasm for the level of resistance against chickpea wilt complex during two years of experimentation.

Based on two years of data, the final disease reaction has been worked out. The final reaction indicated that only two varieties/germplasm *viz.*, Gujarat Junagadh Gram 6 and GAG0624 showed a resistant reaction while three varieties/ germplasm *viz.*, Gujarat Junagadh Gram 3, Dayadara and Khedbrahma showed a moderately resistant reaction.

The 20 varieties/germplasm *viz.*, Gujarat Gram 4, Gujarat Gram 5, Arva, Puhri, Varantha, Godhara nazik, Saliya, Potiya zol, Kheroj, Pavi, Ghelvat, Khakhariya, ACP-21, GAG 1107, GAG 1423, CSJ 740, H 14-22, ACP 1070, ACP 1075 and ACP 1076 showed as moderately susceptible and twelve varieties/germplasm *viz.*, Gujarat Gram 1, Gujarat Gram 2, JG 62, Bavsar, Bagodara, Rayka, Baspa, Kheralu, Vitthalgadh, Mulava, CSJ 882 and ACP 1071 were as susceptible to the disease.

In the present studies, varieties/ germplasm *viz.*, Gujarat Junagadh Gram 6 and GAG0624 were identified as resistant which can be further exploited in resistance breeding programme. A similar type of results was reported by earlier workers *viz.,* Pande *et al.* (2007), Amule *et al.* (2014), Wagh *et al.* (2018), Sharma *et al.* (2019) and Talekar *et al.* (2021) against soil-borne pathogens of chickpea.

Biochemical analysis

The biochemical variation was estimated from diseased and healthy plant roots from two each of resistant (GJG 6and GAG 0624) and susceptible (JG 62 and Bagodara) varieties/ germplasm (Table 3 to 6).

Moisture

Healthy roots of both germplasm contained a higher amount of moisture per cent than diseased roots (Table 3). The moisture content was higher (88.42 and 87.53%) in healthy roots of resistant germplasm *i.e.* GJG 6 and GAG 0624 and 85.90 and 86.21% in susceptible germplasm (JG 62 and Bagodara) followed by diseased roots (80.22 and 77.18%) of resistant germplasm and 74.71 and 75.52% of susceptible germplasm, respectively. So, the moisture content was higher in roots of healthy variety/germplasm than diseased roots of both groups of variety/germplasm.

Protein content

The result revealed that protein content was higher in healthy (GJG 6: 5.74; GAG 0624: 5.58%) as well as diseased (GJG 6: 3.92; GAG 0624: 3.76%) roots of resistant germplasm than healthy (JG 62: 3.60; Bagodara: 3.33%) and diseased (JG 62: 2.32; Bagodara: 1.72%) roots of susceptible germplasm. The least rate of decrease over healthy 32.14%

Table 2: Reaction of different chickpea varieties/germplasm to wilt complex under pot condition.

Varieties/ germplasm	*Rabi* 2018-19				*Rabi* 2019-20				Final disease reaction
	Germination count (Out of 20)	Germination (%)	Seedling mortality (%)	Disease reaction	Germination count (Out of 20)	Germination (%)	Seedling mortality (%)	Disease reaction	
Gujarat gram 1	19	95	26.32	MS	19	95	42.11	S	S
Gujarat gram 2	19	95	36.84	MS	18	90	44.44	S	S
Gujarat Junagadh gram 3	18	90	16.67	MR	18	90	16.67	MR	MR
Gujarat gram 4	19	95	31.58	MS	18	90	33.33	MS	MS
Gujarat gram 5	20	100	15.00	MR	19	95	26.32	MS	MS
Gujarat Junagadh gram 6	20	100	5.00	R	20	100	10.00	R	R
JG 62 (National check)	20	100	70.00	S	19	95	78.95	S	S
Bavsar	19	95	42.11	S	19	95	52.63	S	S
Bagodara	20	100	60.00	S	19	95	73.68	S	S
Rayka	17	85	41.18	S	18	90	44.44	S	S
Baspa	16	80	43.75	S	16	80	50.00	S	S
Arva	20	100	20.00	MS	19	95	21.05	MS	MS
Puhri	20	100	25.00	MS	20	100	30.00	MS	MS
Dayadara	20	100	15.00	MR	20	100	15.00	MR	MR
Varantha	20	100	25.00	MS	19	95	31.58	MS	MS
Godhara nazik	20	100	30.00	MS	19	95	31.58	MS	MS
Saliya	20	100	20.00	MR	20	100	25.00	MS	MS
Kheralu	13	65	30.77	MS	13	65	46.15	S	S
Potiya zol	17	85	17.65	MR	18	90	27.78	MS	MS
Kheroj	18	90	27.78	MS	17	85	29.41	MS	MS
Pavi	19	95	26.32	MS	18	90	38.89	MS	MS
Ghelvat	18	90	11.11	MR	17	85	23.53	MS	MS
Vitthalgadh	16	80	56.25	S	16	80	50.00	S	S
Khedbrahma	16	80	18.75	MR	16	80	18.75	MR	MR
Khakhariya	16	80	18.75	MR	15	75	26.67	MS	MS
Mulava	17	85	58.82	S	16	80	62.50	S	S
GAG-0624	20	100	10.00	R	15	75	6.67	R	R
ACP-21	20	100	25.00	MS	19	95	31.58	MS	MS
GAG 1107	17	85	23.53	MS	18	90	33.33	MS	MS
GAG 1423	19	95	26.32	MS	18	90	27.78	MS	MS
CSJ 740	16	80	18.75	MR	15	75	26.67	MS	MS
H 14-22	17	85	23.53	MS	17	85	35.29	MS	MS
CSJ 882	16	80	43.75	S	15	75	60.00	S	S
ACP 1070	16	80	12.50	MR	15	75	26.67	MS	MS
ACP 1071	17	85	35.29	MS	17	85	47.06	S	S
ACP 1075	17	85	29.41	MS	18	90	27.78	MS	MS
ACP1076	20	100	20.00	MR	19	95	26.32	MS	MS

Note: 0-10%=R, 10.1-20%=MR, 20.1-40%=MS, 40.1-100%=S.

Final disease reaction	No. of variety /germplasm	Variety/germplasm
R	02	Gujarat Junagadh Gram 6 and GAG-0624
MR	03	Gujarat Junagadh Gram 3, Dayadara and Khedbrahma
MS	20	Gujarat Gram 4, Gujarat Gram 5, Arva, Puhri, Varantha, Godhara nazik, Saliya, Potiya zol, Kheroj, Pavi, Ghelvat,Khakhariya, ACP21, GAG 1107, GAG 1423, CSJ 740, H 14-22, ACP 1070, ACP 1075 and ACP 1076
S	12	Gujarat Gram 1, Gujarat Gram 2, JG 62, Bavsar, Bagodara, Rayka, Baspa, Kheralu,Vitthalgadh, Mulava, CSJ 882 and ACP 1071
Total	37	

was recorded in resistant germplasm, whereas it was highest 41.82% in susceptible germplasm (Table 4).

Protein in plant response to adverse conditions is very important as proteins are an indispensable component of innate immune responses in plants under biotic or abiotic stress conditions. Saxena and Karan (1991) and Ushamalini *et al.* (1998) also reported a decline in the protein content of sesame and sunflower seeds artificially inoculated with *F. moniliforme, M. phaseolina, Alternaria alternata, Aspergillus flavus* and *A. niger.*

Phenol content

The result presented in Table 5 revealed that healthy roots of resistant germplasm (GJG 6: 0.62; GAG 0624: 0.61 mg/g fresh weight) contained a higher amount of total phenol than susceptible one (JG 62: 0.51; Bagodara: 0.51 mg/g fresh weight).

Table 3: Effect of wilt complex pathogens on moisture content in resistant and susceptible varieties/germplasm of chickpea roots.

Varieties/ germplasm	Moisture content (%)	
	Healthy root	Diseased root
Resistant		
GJG 6	88.42	80.22
GAG 0624	87.53	77.18
Susceptible		
JG 62	85.90	74.71
Bagodara	86.21	75.52
S.Em.±	0.44	0.63
C. D. at 5%	1.34	1.97
C.V.%	1.00	1.66

The phenol amount was increased in resistant and susceptible diseased roots, but the higher increase of phenol was observed in resistant germplasm, while it was at low insusceptible germplasm. The result revealed that per cent increase in total phenol was higher (27.95%) in resistant germplasm, while it was low (25.14%) in susceptible germplasm.

Phenol is known to impart resistance against pathogens, because of their antimicrobial activity. It is often assumed that their main role in plants is to act as a protective compound against disease caused by fungi, bacteria and viruses. The higher amount of phenolic compounds in diseased roots of chickpea germplasm may be due to several factors including either enhancement of synthesis or translocation of phenolic to the site of infection. These findings are in harmony with the similar studies carried out on activity of phenol changes due to pathogen infection by Rathod and Vakharia (2011), Belkar *et al.* (2018) and Jyothi *et al.* (2018). They also found an increase in the activity of phenol in chickpea due to infection of *F. oxysporum* f. sp. *ciceri* and *M. phaseolina*.

Total soluble sugars

Higher amount of total soluble sugars content were presented in healthy roots of resistant germplasm (GJG 6: 5.09; GAG 0624: 4.94 mg/g fresh weight) than susceptible germplasm (JG 62: 3.19; Bagodara: 2.95 mg/g fresh weight). The least rate of decrease (32.14%) was recorded in resistant germplasm, whereas the highest rate of decrease (41.82%) was recorded in susceptible germplasm (Table 6).

Sugars play an important role in the inhibition of pectinolytic and cellulolytic enzymes, which are essential

Table 4: Effect of wilt complex pathogens on protein content in resistant and susceptible varieties/germplasm of chickpea roots.

Type of root	Protein content (%)									
	Resistant					Susceptible				
	GJG 6	GAG 0624	Total	Mean	DoH(%)	JG 62	Bagodara	Total	Mean	DoH(%)
Healthy	5.74	5.58	11.32	5.66	32.14	3.60	3.33	6.94	3.47	41.82
Diseased	3.92	3.76	7.68	3.84		2.32	1.72	4.04	2.02	

Note: DoH- Decrease over healthy.

Table 5: Effect of wilt complex pathogens on phenol contentin resistant and susceptible varieties/germplasm of chickpea roots.

Type of root	Phenol content (mg/g fresh weight)									
	Resistant					Susceptible				
	GJG 6	GAG 0624	Total	Mean	IoH(%)	JG 62	Bagodara	Total	Mean	IoH(%)
Healthy	0.62	0.61	1.23	0.62	27.95	0.51	0.51	1.01	0.51	25.14
Diseased	0.87	0.84	1.71	0.85		0.68	0.68	1.35	0.68	

Note: IoH- Increase over healthy.

Table 6: Effect of wilt complex pathogens on total soluble sugars content in resistant and susceptible varieties/germplasm of chickpea roots.

Type of root	Total soluble sugars content (mg/g fresh weight)									
	Resistant					Susceptible				
	GJG 6	GAG 0624	Total	Mean	DoH(%)	JG 62	Bagodara	Total	Mean	DoH(%)
Healthy	5.09	4.94	10.03	5.01	32.14	3.19	2.95	6.14	3.07	41.82
Diseased	3.47	3.33	6.80	3.40		2.05	1.52	3.57	1.79	

Note: DoH- Decrease over health.

for the pathogen. Moreover, sugars are precursors of phenolics, which are highly toxic to germplasm. Results similar to the present investigations were observed by Ushamalini *et al.* (1998), Belkar *et al.* (2018) and Jyothi *et al.* (2018) as they reported high sugars content in resistant genotypes compared to susceptible genotypes.

CONCLUSION

Among the thirty-seven chickpea varieties/ germplasm screened, two lines *viz.,* Gujarat Junagadh Gram 6 and GAG0624 can be used for wilt complex resistance breeding program based on their resistance reaction. Further, biochemical constituents revealed that the diseased plant roots had a lower percentage of moisture, protein and total soluble sugars over the healthy plant roots whereas, the phenol content was higher in the diseased plant roots.

ACKNOWLEDGEMENT

Authors gratefully acknowledge Agricultural Research Station, Anand Agricultural University, Arnej and Pulse Research Station, Junagadh Agricultural University, Junagadh for providing screening material.

Conflict of interest: None.

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